THE EFFECT OF VASOACTIVE ANTAGONISTS IN ENDOTOXIN SHOCK

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The effect of vasoactive antagonists in endotoxin shock

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Injection of lethal quantities of gram-negative bacterial endotoxin into the circulation of dogs results in characteristic vascular changes during the ensuing 30-minute period, including development of systemic arterial hypotension, portal venous hypertension, and an increase in splanchnic vascular resistance.[1] Several naturally occurring vasoactive substances have been implicated in the pathogenesis of endotoxin shock. These include the catecholamines,[2] histamine,[3-7] serotonin,[8,9] and bradykinin.[10]

A possible role for the sympathetic nervous system in endotoxin shock was first suggested by Reilly and associates,[11] who showed that direct injection of minute quantities of endotoxin into splanchnic nerves or autonomic ganglia induced gastrointestinal hemorrhages and necrosis, shock and death. Penner[12] demonstrated that tetraethylammonium chloride and ergotamine tartrate were able to abort the intestinal lesions induced by endotoxin. Other reports showed that Dibenamine[13,14] prevented the increased vasomotion of endotoxemia.

More recent studies have shown that spinal-cord section does not prevent the hemodynamic changes observed early in endotoxin shock.[15] This has resulted in a shift in emphasis from the sympathetic nervous system to the circulating catecholamines. This concept is supported by the work of Zweifach and associates[2] and Gourzis and associates,[16] who have observed a heightened vascular sensitivity to pressor catecholamines during endotoxemia. Furthermore, regional studies of perfused stomach,[17] small intestine,[18] spleen,[19] kidney,[20,21] and lung[22] indicate that endotoxin increases vascular resistance in these organs, and that phentolamine[17,21] can block this rise in resistance. In addition, catecholamine antagonists have been shown to increase survival in endotoxin shock.[16,23]

Other investigators have focused attention on histamine as the primary agent involved in the vascular events of endotoxin shock. Schayer and his collaborators[4,5] have shown that both endotoxin and the histamine liberator, Compound 48/80, enhance histidine decarboxylase activity. Hinshaw and associates[7] have described certain similarities in the hemodynamic responses of the dog to histamine, Compound 48/80, and endotoxin.

The dramatic decline in the circulating concentrations of 5-hydroxytryptamine (serotonin) during endotoxin shock,[9] and the protective action of serotonin in endo-

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toxemia[8] suggest an important role for this agent.

This investigation describes the early hemodynamic effects of endotoxin in dogs whose response to catecholamines, histamine, acetylcholine, or serotonin was altered pharmacologically.

Methods and materials

Seventy-five mongrel dogs of both sexes which weighed from 10 to 29 kilograms were the subjects of these experiments. Sixty of these animals were anesthetized with pentobarbital sodium (30 mg. per kilogram) administered intravenously. An endotracheal tube was inserted and connected to a positive-pressure respirator* with a respiratory minute volume of 2,500 to 4,000 c.c. per minute, which maintains a normal pH and pCO_2, thus obviating altered vascular responses to either endotoxin[24] or catecholamines.[25] A left subcostal laparotomy was performed, and the splenic artery was exposed. Those branches of the splenic artery which passed to the stomach and the pancreas were ligated. Heparin sodium (10 mg. per kilogram) was injected intravenously, and polyethylene tubing was connected through a finger pump† to a metal cannula which was inserted into the splenic artery. Blood flow to the spleen was interrupted for less than 1 minute by this procedure. Flow was fixed in the pump so that pressure in the tubing proximal to the splenic artery would approximate mean systemic arterial pressure recorded from the left femoral artery in 60 animals (Dogs 1-45, 51-65, Table I). In 5 animals (Dogs 61-65), systemic arterial pressure was intentionally reduced 50 per cent by exsanguination. In 5 animals (Dogs 46-50), splenic arterial pressure was purposely maintained at a pressure approximately 50 per cent below systemic arterial pressure.

In 5 animals (Dogs 21-25), morphine sulfate (15 mg. subcutaneously) and chloralose (75 mg. per kilogram intravenously) were employed to induce anesthesia with agents that would not have atropine-like effects, and the surgical procedure outlined above was performed.

These 65 animals were divided into 13 groups of 5 dogs each, arranged in Table I according to pharmacologic pretreatment, systemic arterial and splenic arterial pressures, or anesthesia. The agents used prior to the injection of endotoxin included the following:

1. Reserpine (CIBA Pharmaceutical Co., Summit, N. J.), 0.2 mg. per kilogram injected intravenously 2 hours before and repeated 1 hour before endotoxin in 5 dogs.

2. Compound 48/80 (Burroughs, Wellcome Co., Inc., Tuckahoe, N. Y.), 0.1 mg. per kilogram injected intraperitoneally twice daily for 5 days before endotoxin in 5 dogs. One hour before endotoxin was administered, an additional 0.5 mg. per kilogram was infused intravenously.

3. Atropine sulfate, 0.4 mg. per kilogram injected intravenously 10 minutes before endotoxin. This dose blocked the systemic arterial depressor response to 300 μg of acetylcholine.

4. Pyrilamine maleate (Merck & Co., Inc., West Point, Pa.), 50 mg. per kilogram injected intravenously over a 2-hour period prior to endotoxin. This drug failed to attenuate the systemic arterial depressor response to 100 μg of histamine or the pressor response to 100 μg of norepinephrine administered intravenously. Pyrilamine maleate was used in only 1 dog.

5. Diphenhydramine hydrochloride (Benadryl, Parke, Davis & Co., Detroit, Mich.), 50 mg. per kilogram injected intravenously over a 1-hour period prior to endotoxin. This dose abolished the depressor response to 10 μg of histamine and markedly attenuated the depressor response to 36 and 100 μg of histamine injected intravenously. One animal was pretreated with this drug.

6. N-(2′dimethylamino-2′methyl) ethyl phenothiazine hydrochloride (Phenergan, Wyeth Laboratories, Philadelphia, Pa.), 50 mg. per kilogram injected intravenously over a 1-hour period prior to endotoxin in 3 dogs. This drug abolished the depressor response to 10 μg and markedly attenuated the depressor response to 36 and 100 μg of histamine injected intravenously. The depressor response to 33 μg of isoproterenol was also attenuated, but the depressor response to 100 μg of isoproterenol was unaffected by this phenothiazine derivative. In one of these animals, diphenhy-

*Harvard Apparatus Co., Dover, Mass.
†Sigmamotor, Inc., Middleport, N. Y.

Table I. *Division of dogs into 13 groups according to pharmacologic pretreatment, anesthesia, and pressures in the systemic and perfusion circuits prior to injection of endotoxin*

Group	*Dog numbers*	*Anesthesia*	*Mean splenic flow (ml./min.)*	*Mean pressures (mm. Hg)*		*Pharmacologic pretreatment*
				Systemic artery	*Splenic artery*	
I.	1–5	Pentobarbital	38	99	110	Control—No agent
II.	6–10	Pentobarbital	36	80	94	Reserpine
III.	11–15	Pentobarbital	26	83	106	Compound 48/80
IV.	16–20	Pentobarbital	40	101	104	Atropine
V.	21–25	Morphine and chloralose	41	90	100	Control—No agent
VI.	26–30	Pentobarbital	33	70	91	Antihistaminics
VII.	31–35	Pentobarbital	29	60	78	Cyproheptadine
VIII.	36–40	Pentobarbital	34	104	114	Control—Solvent for phenoxybenzamine
IX.	41–45	Pentobarbital	30	45	65	Phenoxybenzamine
X.	46–50	Pentobarbital	14	109	65	Control—Low perfusion pressure
XI.	51–55	Pentobarbital	26	66	66	Nethalide
XII.	56–60	Pentobarbital	35	72	74	DCI
XIII.	61–65	Pentobarbital	8	51	74	Control—Low systemic artery

dramine hydrochloride, 25 mg. per kilogram, was also administered.

7. 1-Methyl-4-(5 dibenso [a, e] -cycloheptatrienylidene)-piperidine (Cyproheptadine, Merck & Co., Inc., West Point, Pa.), 15 to 25 mg. per kilogram injected intravenously over a 1-hour period prior to endotoxin. This drug was dissolved in acetic acid and saline (pH 4.0). In these doses this agent abolished or markedly attenuated the depressor response to 40 and 100 μg of serotonin, and markedly attenuated the pressor response to 200 and 400 μg of serotonin injected intravenously in 5 dogs. This serotonin antagonist also converted the depressor response of 33 μg of isoproterenol to a pressor response, markedly attenuated the depressor response to 36 μg of histamine, but had no effect on the pressor response to 33 μg of l-norepinephrine.

8. Phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French Laboratories, Philadelphia, Pa.) 20 mg. per kilogram injected intravenously over a 1-hour period prior to endotoxin in 5 dogs. This substance was dissoved in a solution containing propylene glycol and acetic acid (pH 4.0). In these doses, phenoxybenzamine abolished the pressor response to 100 μg of l-norepinephrine, and somewhat attenuated the depressor response to 100 μg of histamine.

9. 2 (D-hydroxy-3-isopropyl aminoethyl naphthalene) (Nethalide, Ayerst Laboratories, New York, N. Y.), 30 mg. per kilogram injected intravenously over a 1½-hour period prior to endotoxin. In these doses, Nethalide markedly attenuated or abolished the depressor response to 100 μg of isoproterenol, incompletely reduced the depressor response to 100 μg of histamine, and caused some reduction in the pressor responses to 100 μg of l-norepinephrine and serotonin. Nethalide was administered to 5 dogs.

10. 1-(3′, 4′-Dichlorophenyl)-2-isopropylaminoethanol hydrochloride (Aldrich Chemical Co., Inc., Milwaukee, Wisc.) or DCI, 40 to 80 mg. per kilogram injected intravenously over a 1-hour period prior to endotoxin in 5 dogs. In these doses, DCI abolished the depressor response to 33 μg of isoproterenol, enhanced the pressor response to 100 μg of l-norepinephrine, somewhat diminished the depressor response to 100 μg of histamine and the pressor response to 400 μg of serotonin.

The lethality of the single lot of endotoxin (*Shigella flexneri* 2A*) employed in these experiments was determined in an additional 10 unanesthetized dogs after preliminary lethality studies in guinea pigs. Half of the animals were killed within 24 hours by a single intravenous injection of 0.6 mg. per kilogram. An LD_{50} was employed in these experiments rather than the frequently used endotoxin doses which are many times greater than an LD_{100}, for two reasons: the effectiveness of any potential pharmacologic agent in opposing some hemodynamic action of endotoxin could be entirely overwhelmed by massive doses of endotoxin.

After a period of approximately 30 minutes of perfusion of the spleen to allow hemodynamic stabilization, endotoxin was injected into the splenic artery, and mean pressures were monitored in the splenic artery, a mesenteric branch of the portal vein, and the left femoral artery (systemic arterial pressure) by means of a strain-gauge transducer connected to a recorder.† Splenic vascular resistance was calculated as the quotient of the mean pressure gradient from the perfused splenic artery to the portal vein and the constant flow, and was expressed as millimeters of mercury per milliliter per minute (P.R.U.). Pressures were obtained at 1-minute intervals for 30 minutes after injection of endotoxin. The well-described "first phase" of endotoxin shock in the dog is complete within this period of time and offers an opportunity of observing the effects of neurohumoral blocking agents on a complex but reproducible hemodynamic entity. The results of a typical experiment are shown in Fig. 1.

It should be noted that we observed certain consistent differences from the usual pattern of changes in pressure which has been reported elsewhere.[1] The increase in portal pressure and the decline in systemic arterial pressure after injection of an LD_{50} of *Shigella flexneri* endotoxin are neither as abrupt nor as profound as those observed with fully lethal doses of *Escherichia coli* endotoxin.

The vascular responses to endotoxin in pharmacologically pretreated dogs were compared by noting the number of animals in each group whose systemic arterial or splenic perfusion pressures changed by more than 10 mm. Hg, or whose portal venous pressures increased by at least 4 mm. Hg during the 30-minute period of observation. In addition, statistical comparison of the slopes of pressure and resistance as functions of time during endotoxin shock with or without the various pharmacologic or mechanical treatments was performed, employing an analysis of combined hierarchial design[26] which was programmed for a computer.*

Results

Control. The injection of 0.6 mg. per kilogram of the endotoxin of *Shigella*

*Difco Laboratories, Detroit, Mich.
†Sanborn Co., Waltham, Mass.
*RPC 4000, Royal Precision Co., Bethesda, Md.

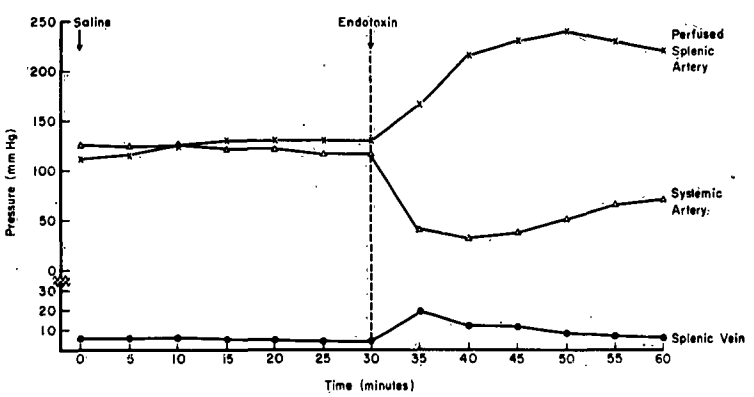

Fig. 1. Typical responses of perfused splenic arterial, systemic arterial, and splenic venous mean pressures to the injection of saline and endotoxin. (U. S. Army photograph.)

flexneri, type 2A, into the perfusion circuit of 5 control dogs resulted in portal venous hypertension, systemic arterial hypotension, and an increase in both perfusion pressure and splenic vascular resistance. Mean pressure in the portal vein doubled 5 minutes after endotoxin had been administered, pressure in the femoral artery decreased 22 per cent at 10 minutes and 17 per cent at 30 minutes after endotoxin, perfusion pressure increased 36 per cent, and the resistance to the flow of blood

Table II. *Comparison of changes in systemic arterial pressure, splenic arterial pressure, portal in 6 groups of 5 dogs each**

Group	*Time (minutes)*			
	0	*5*	*10*	*15*
	Systemic arterial pressure (mm. Hg)			
I. Control	99 ± 5	84 ± 11	78 ± 13	78 ± 12
II. Reserpine	80 ± 4	50 ± 7	53 ± 4	46 ± 6
III. 48/80	83 ± 13	72 ± 8	59 ± 12	57 ± 12
IV. Atropine	101 ± 7	80 ± 5	83 ± 7	79 ± 5
V. Morphine and chloralose	90 ± 12	76 ± 11	57 ± 14	52 ± 13
VI. Antihistaminics	70 ± 7	53 ± 5	49 ± 5	52 ± 4
	Splenic arterial pressure (mm. Hg)			
I. Control	110 ± 7	121 ± 9	140 ± 6	149 ± 11
II. Reserpine	94 ± 5	119 ± 9	140 ± 19	133 ± 15
III. 48/80	106 ± 7	117 ± 6	123 ± 7	129 ± 12
IV. Atropine	104 ± 11	117 ± 10	131 ± 9	134 ± 10
V. Morphine and chloralose	100 ± 4	122 ± 8	150 ± 17	160 ± 16
VI. Antihistaminics	91 ± 8	105 ± 7	110 ± 4	112 ± 6
	Portal venous pressure (mm. Hg)			
I. Control	7 ± 1	14 ± 2	11 ± 1	8 ± 1
II. Reserpine	6 ± 1	12 ± 1	10 ± 2	7 ± 2
III. 48/80	7 ± 0	10 ± 1	8 ± 1	7 ± 1
IV. Atropine	6 ± 1	11 ± 2	9 ± 1	7 ± 1
V. Morphine and chloralose	6 ± 1	15 ± 2	12 ± 2	10 ± 1
VI. Antihistaminics	8 ± 1	15 ± 1	11 ± 1	9 ± 1
	Splenic vascular resistance (P.R.U.)			
I. Control	3.20 ± .79	3.30 ± .87	3.89 ± .81	4.31 ± .97
II. Reserpine	2.72 ± .52	3.27 ± .57	3.98 ± .91	3.86 ± .79
III. 48/80	3.94 ± .47	4.31 ± .57	4.67 ± .72	5.00 ± .94
IV. Atropine	2.70 ± .47	2.97 ± .50	3.44 ± .54	3.58 ± .61
V. Morphine and chloralose	2.77 ± .59	3.25 ± .74	4.03 ± .88	4.37 ± .95
VI. Antihistaminics	2.77 ± .67	2.97 ± .65	3.24 ± .63	3.37 ± .68

*The groups are divided according to treatment before endotoxin. p is the probability of no difference in the time sequence of changes arterial pressure declined or splenic arterial pressure increased more than 10 mm. Hg, or portal venous pressure increased by at

across the spleen increased 35 per cent at 15 minutes after the injection of endotoxin. These results appear in Table II.

Reserpine. The 5 animals pretreated with reserpine exhibited hemodynamic responses to endotoxin which were similar to those of the control group (Table II). Portal venous pressure increased 100 per cent in 5 minutes, systemic arterial pressure declined 34 per cent in 10 minutes, perfusion pressure climbed 49 per cent, and splenic vascular resistance rose 42 per cent in 15

venous pressure, and splenic vascular resistance in response to endotoxin injected at zero time

Time (minutes)—Cont'd			*N.P.G.*	*p*
20	*25*	*30*		
Systemic arterial pressure (mm. Hg)				
79 ± 10	82 ± 8	82 ± 7	5/5	—
47 ± 7	48 ± 9	46 ± 11	5/5	n.s.
54 ± 11	51 ± 11	45 ± 12	5/5	< .01
75 ± 5	76 ± 4	79 ± 5	4/5	n.s.
52 ± 12	53 ± 13	56 ± 15	5/5	< .05
50 ± 4	51 ± 5	51 ± 4	4/5	n.s.
Splenic arterial pressure (mm. Hg)				
145 ± 10	145 ± 11	147 ± 14	5/5	—
133 ± 16	135 ± 20	137 ± 22	5/5	n.s.
131 ± 16	130 ± 18	130 ± 21	4/5	n.s.
132 ± 10	130 ± 10	129 ± 10	5/5	< .05
158 ± 17	158 ± 18	161 ± 18	5/5	n.s.
108 ± 8	104 ± 9	104 ± 11	5/5	< .001
Portal venous pressure (mm. Hg)				
8 ± 1	7 ± 0	7 ± 1	5/5	—
6 ± 1	7 ± 2	7 ± 2	3/5	n.s.
7 ± 1	6 ± 1	6 ± 1	1/5	< .01
6 ± 1	5 ± 1	5 ± 1	3/5	n.s.
7 ± 2	6 ± 1	6 ± 2	5/5	n.s.
8 ± 1	7 ± 1	7 ± 0	5/5	n.s.
Splenic vascular resistance (P.R.U.)				
4.03 ± .76	4.23 ± .98	4.25 ± .98		—
3.89 ± .79	3.92 ± .84	4.03 ± .95		n.s.
5.16 ± 1.12	5.16 ± 1.18	5.15 ± 1.30		n.s.
3.51 ± .58	3.50 ± .59	3.51 ± .61		< .05
4.41 ± .94	4.50 ± 1.04	4.59 ± 1.03		n.s.
3.31 ± .75	3.24 ± .78	3.20 ± .79		n.s.

in pressure or resistance between each group and control. N.P.G. signifies the number of animals per group of 5 in which systemic least 4 mm. Hg within 30 minutes after endotoxin. The numbers tabulated are mean values ± standard error of the mean.

minutes after endotoxin had been injected.

Compound 48/80. Animals pretreated with Compound 48/80 showed a somewhat less marked response of portal venous pressure and a somewhat greater and more sustained systemic hypotensive response to endotoxin (Table II). Thus, portal venous pressure rose 43 per cent at 5 minutes, systemic arterial pressure fell 29 per cent at 10 minutes and 45 per cent at 30 minutes, and perfusion pressure increased 21 per cent at 15 minutes after the injection of endotoxin.

Atropine. In 5 dogs pretreated with atropine the effects of endotoxin on systemic arterial and portal pressures were not different from those in the control series (Table II). Peak mean portal venous pressure was observed 5 minutes after injection of endotoxin (+83 per cent), systemic arterial pressure declined 20 per cent at 5 minutes after endotoxin and was 17 per cent lower than preinjection values at 10 minutes after endotoxin. Perfusion pressure and splenic vascular resistance exhibited a somewhat less marked increase in response to endotoxin; however, in all 5 animals an increase in perfusion pressure of more than 10 mm. Hg was noted within 30 minutes after endotoxin.

Morphine and chloralose. Since pentobarbital has an anticholinergic effect, 5

*Table III. Comparison of changes in systemic arterial pressure, splenic arterial pressure, portal in 4 groups of 5 dogs each**

Group	Time (minutes)		
	0	5	10
Systemic arterial pressure (mm. Hg)			
VIII. Control-solvent	104 ± 14	89 ± 19	86 ± 13
VII. Cyproheptadine	60 ± 9	50 ± 6	52 ± 8
IX. Phenoxybenzamine	45 ± 5	39 ± 4	33 ± 3
X. Low perfusion pressure	109 ± 1	82 ± 5	85 ± 5
Splenic arterial pressure (mm. Hg)			
VIII. Control-solvent	114 ± 8	122 ± 10	135 ± 7
VII. Cyproheptadine	78 ± 9	86 ± 9	85 ± 9
IX. Phenoxybenzamine	65 ± 8	68 ± 10	70 ± 11
X. Low perfusion pressure	65 ± 7	74 ± 6	94 ± 11
Portal venous pressure (mm. Hg)			
VIII. Control-solvent	6 ± 2	15 ± 3	12 ± 1
VII. Cyproheptadine	10 ± 0	13 ± 1	11 ± 1
IX. Phenoxybenzamine	6 ± 1	6 ± 1	6 ± 1
X. Low perfusion pressure	7 ± 1	15 ± 0	11 ± 1
Splenic vascular resistance (P.R.U.)			
VIII. Control-solvent	3.74 ± .87	3.85 ± .88	4.22 ± .87
VII. Cyproheptadine	2.60 ± .63	2.77 ± .59	2.80 ± .60
IX. Phenoxybenzamine	2.06 ± .35	2.15 ± .38	2.20 ± .39
X. Low perfusion pressure	4.08 ± .24	4.15 ± .41	5.98 ± .76

*The groups are divided according to treatment before endotoxin. The control group received an infusion of the solvent used to dissolve substance between each group and control. N.P.G. signifies the number of animals per group of 5 in which systemic arterial pressure within 30 minutes after endotoxin. The numbers tabulated are mean values ± standard error of the mean.

animals were anesthetized with morphine and chloralose for purposes of comparison with the control and atropine-treated groups. The injection of endotoxin in these dogs produced hemodynamic events similar to those of either control or atropine-treated dogs (Table II). Portal venous pressure increased 150 per cent at 5 minutes and was still 100 per cent above preinjection pressures at 10 minutes after injection. Pressure in the splenic artery increased 60 per cent and vascular resistance rose 58 per cent at 15 minutes after endotoxin was administered. Systemic arterial pressure declined 40 per cent at 15 minutes after endotoxin.

Antihistaminics. Pretreatment of 5 dogs with sizeable doses of antihistaminics did not alter the pattern of systemic or portal hemodynamic responses but reduced somewhat the perfusion pressure response to endotoxin (Table II). Portal venous pressure increased 87 per cent at 5 minutes, systemic arterial pressure declined 30 per cent at 10 minutes, splenic arterial pressure increased 23 per cent and splenic vascular resistance increased 20 per cent at 15 minutes after endotoxin was injected. However, all 5 animals exhibited an increase in splenic arterial pressure of 10 mm. Hg or more during the period of observation.

venous pressure, and splenic vascular resistance in response to endotoxin injected at zero time

Time (minutes)—Cont'd				*N.P.G.*	*p*
15	*20*	*25*	*30*		
Systemic arterial pressure (mm. Hg)					
87 ± 12	86 ± 15	83 ± 15	85 ± 13	5/5	—
50 ± 7	46 ± 6	43 ± 6	40 ± 6	5/5	n.s.
31 ± 3	29 ± 4	25 ± 3	20 ± 4	5/5	< .05
89 ± 5	87 ± 3	89 ± 3	90 ± 3	5/5	n.s.
Splenic arterial pressure (mm. Hg)					
150 ± 10	152 ± 12	147 ± 12	147 ± 11	5/5	—
81 ± 8	76 ± 9	75 ± 10	73 ± 9	1/5	< .001
69 ± 10	63 ± 9	58 ± 7	55 ± 7	1/5	< .001
95 ± 11	86 ± 11	83 ± 10	84 ± 10	4/5	n.s.
Portal venous pressure (mm. Hg)					
9 ± 1	7 ± 1	7 ± 1	6 ± 1	3/5	—
10 ± 1	8 ± 1	8 ± 1	7 ± 1	3/5	n.s.
5 ± 1	5 ± 1	4 ± 1	3 ± 1	0/5	< .001
9 ± 1	8 ± 1	8 ± 0	8± 1	5/5	n.s.
Splenic vascular resistance (P.R.U.)					
4.80 ± .99	4.91 ± .98	4.67 ± .81	4.75 ± .87		—
2.72 ± .63	2.62 ± .66	2.64 ± .77	2.58 ± .74		< .001
2.19 ± .38	2.02 ± .34	1.88 ± .31	1.78 ± .28		< .001
6.11 ± .69	5.53 ± .69	5.42 ± .78	5.49 ± .80		n.s.

phenoxybenzamine and Cyproheptadine. p is the probability of no difference in the time sequence of changes in pressure or re-
declined or splenic arterial pressure increased more than 10 mm. Hg, or portal venous pressure increased by at least 4 mm. Hg

Cyproheptadine. The use of Cyproheptadine in 5 dogs was attended by a marked decrease in the magnitude of changes in splenic arterial pressure after the injection of endotoxin. The increases in splenic arterial pressure and splenic vascular resistance were 10 and 7 per cent, respectively, at 5 minutes, and 9 and 8 per cent, respectively, at 10 minutes after endotoxin. Systemic arterial pressure had declined 16 per cent from preinjection values at 5 minutes, 13 per cent at 10 minutes, and 33 per cent at 30 minutes after endotoxin. These results appear in Table III.

Control-solvent. The 5 dogs pretreated with the solvent used as a vehicle for phenoxybenzamine and Cyproheptadine exhibited hemodynamic alterations quite similar to those of the first control group (Table III).

Phenoxybenzamine. Prior infusion of phenoxybenzamine induced marked alteration of the splanchnic vascular responses to endotoxin (Table III). There was no increase in mean portal venous pressure, and only an 8 per cent rise in perfusion pressure and a 7 per cent increase in vascular resistance at any time during the 30-minute period of observation. The pattern of response in these dogs was significantly different from that of control animals ($p = < .001$ for either of the pres-

*Table IV. Comparison of changes in systemic arterial pressure, splenic arterial pressure, portal in 4 groups of 5 dogs each**

Group	Time (minutes)			
	0	5	10	15
	Systemic arterial pressure (mm. Hg)			
I. Control	99 ± 5	84 ± 11	78 ± 13	78 ± 12
XI. Nethalide	66 ± 8	60 ± 7	60 ± 8	60 ± 9
XII. DCI	72 ± 4	62 ± 9	67 ± 8	63 ± 7
XIII. Hemorrhage	51 ± 5	43 ± 4	40 ± 5	44 ± 5
	Splenic arterial pressure (mm. Hg)			
I. Control	110 ± 7	121 ± 9	140 ± 6	149 ± 11
XI. Nethalide	66 ± 6	90 ± 10	101 ± 13	91 ± 13
XII. DCI	74 ± 7	118 ± 8	136 ± 9	125 ± 11
XIII. Hemorrhage	74 ± 7	79 ± 6	76 ± 6	76 ± 8
	Portal venous pressure (mm. Hg)			
I. Control	7 ± 1	14 ± 2	11 ± 1	8 ± 1
XI. Nethalide	6 ± 1	11 ± 2	9 ± 1	8 ± 1
XII. DCI	6 ± 0	11 ± 0	9 ± 1	7 ± 1
XIII. Hemorrhage	6 ± 1	9 ± 1	8 ± 1	7 ± 0
	Splenic vascular resistance (P.R.U.)			
I. Control	3.20 ± .79	3.30 ± .87	3.89 ± .81	4.31 ± .97
XI. Nethalide	2.61 ± .63	3.25 ± .54	3.66 ± .50	3.31 ± .49
XII. DCI	2.35 ± .64	3.59 ± .91	4.29 ± 1.01	4.03 ± 1.06
XIII. Hemorrhage	8.53 ± .64	8.79 ± .65	8.78 ± .57	8.85 ± .53

*The groups are divided according to treatment before endotoxin. p is the probability of no difference in the time sequence of changes in arterial pressure declined or splenic arterial pressure increased more than 10 mm. Hg, or portal venous pressure increased by at

sures or resistance). Systemic arterial pressure exhibited a steady decline over the period of observation; at 10 minutes after endotoxin the decrease averaged 27 per cent, and at 30 minutes had reached a level 55 per cent below preinjection pressures. It should be noted, also, that phenoxybenzamine induced profound changes in these animals before endotoxin was administered. Pressures in the perfusion circuit and in the femoral artery were approximately 50 per cent lower than pressures obtained in control dogs before endotoxin was injected.

Low perfusion pressure. In a series of 5 dogs whose perfusion pressures were maintained at levels comparable to pressures in the phenoxybenzamine-treated group before injection of endotoxin, the responses of perfusion pressure and splenic vascular resistance to endotoxin were similar to events observed in control dogs (Table III). However, these splanchnic responses differed significantly from the sequence recorded from dogs treated with phenoxybenzamine ($p = < .001$ for pressure and $< .001$ for resistance). Portal venous and systemic arterial pressures were not significantly different from those of control dogs in this group.

Nethalide. The splanchnic vascular responses to endotoxin in animals pretreated

venous pressure, and splenic vascular resistance in response to endotoxin injected at zero time

Time (minutes)—Cont'd			*N.P.G.*	*p*
20	*25*	*30*		
Systemic arterial pressure (mm. Hg)				
79 ± 10	82 ± 8	82 ± 7	5/5	—
62 ± 9	62 ± 8	62 ± 8	2/5	< .05
61 ± 8	63 ± 7	62 ± 7	2/5	< .05
45 ± 5	42 ± 5	39 ± 5	4/5	< .05
Splenic arterial pressure (mm. Hg)				
145 ± 10	145 ± 11	147 ± 14	5/5	—
86 ± 13	85 ± 13	85 ± 14	4/5	< .001
116 ± 12	112 ± 14	108 ± 14	5/5	< .001
77 ± 9	73 ± 9	72 ± 8	4/5	—
Portal venous pressure (mm. Hg)				
8 ± 1	7 ± 0	7 ± 1	5/5	—
7 ± 1	7 ± 1	6 ± 1	4/5	n.s.
6 ± 1	6 ± 1	6 ± 1	5/5	n.s.
7 ± 0	6 ± 1	6 ± 0	3/5	n.s.
Splenic vascular resistance (P.R.U.)				
4.03 ± .76	4.23 ± .98	4.25 ± .98		—
3.23 ± .54	3.14 ± .59	3.34 ± .86		< .001
3.79 ± 1.06	3.68 ± 1.09	3.56 ± 1.04		< .001
8.75 ± .49	8.27 ± .53	8.28 ± .46		—

pressure or resistance between each group and control. N.P.G. signifies the number of animals per group of 5 in which systemic least 4 mm. Hg within 30 minutes after endotoxin. The numbers tabulated are mean values ± standard error of the mean.

with Nethalide resembled events in control animals, except for a more rapid return of splenic arterial pressure and resistance toward base-line levels. The changes in systemic arterial pressure after injection of endotoxin exhibited a time course similar to that in control animals; however, the magnitude of fall in pressure was considerably reduced in these animals. In the Nethalide group, systemic pressure declined 10 per cent at 10 minutes and was only 6 per cent below starting values at 30 minutes after the injection of endotoxin. By contrast, systemic arterial pressure had fallen 22 per cent at 10 minutes and 10 per cent at 30 minutes after endotoxin in the control dogs. Of the 5 animals pretreated with Nethalide, only 2 manifested a decline in systemic arterial pressure exceeding 10 mm. Hg within 30 minutes after the injection of endotoxin. Of the 15 dogs which could be considered to be controls (Groups I, V and VIII, Table I), all showed a drop in systemic arterial pressure of more than 10 mm. Hg within the 30 minutes after endotoxin. These results appear in Table IV.

DCI. The animals which received the beta receptor antagonist, DCI, exhibited hemodynamic responses to endotoxin similar to those observed in the group given Nethalide (Table IV). Thus, 3 of these dogs did not manifest the early hypotensive response to endotoxin, and the group had a mean fall in systemic arterial pressure of 14 per cent at 5 minutes and 7 per cent at 10 minutes after endotoxin was injected. The perfusion pressure responded to endotoxin in a manner similar to that in animals treated with Nethalide: a marked increase maximal at 10 minutes, followed by a return toward preinjection pressures.

Hemorrhage. Since systemic arterial pressure was materially reduced in many of the groups prior to the administration of endotoxin (Table I), partial exsanguination was employed to provide a hypotensive group for comparison purposes. Systemic arterial pressure fell 22 per cent at 10 minutes after endotoxin in this group (Table IV). Four of these animals exhibited a fall in pressure of more than 10 mm. Hg within 10 minutes after endotoxin was injected.

Discussion

The results of this study indicate that the abrupt increase in portal venous pressure and the rise in splanchnic vascular resistance induced by endotoxin are essentially abolished by prior treatment with an alpha receptor (pressor) antagonist, phenoxybenzamine. In addition, in a majority of dogs, the profound early fall in systemic arterial pressure seen in endotoxin shock was greatly attenuated by prior treatment with the beta receptor (depressor) antagonists, Nethalide and DCI. Splanchnic vascular responses to endotoxin were somewhat modified by prior administration of Cyproheptadine, Compound 48/80, or antihistaminics. There were no consistent changes in the early hemodynamic events of endotoxemia in animals pretreated with reserpine, atropine, or morphine and chloralose.

The nature of this study introduces certain limitations in the interpretation of these findings. The obvious technical shortcomings of this investigation include the use of the dog as the experimental model for endotoxin shock. This animal exhibits hemodynamic responses to endotoxin unlike those of the monkey, rabbit, and cat.[27] The use of deep pentobarbital anesthesia induces a hyporeactive nervous state, particularly with respect to the parasympathetic nervous system. The extensive surgery involved and the use of a mechanical pump which traumatizes formed blood elements add further variables which compromise the physiologic condition of the animal. Finally, the doses of pharmacologic agents required to block specific neurohumoral substances were of such magnitude as to induce toxicity in the animals, to antagonize other neurohumoral substances, and to limit severely any clinical inferences.

Both the attenuation of portal hypertension and the increase in splenic vascular resistance by phenoxybenzamine implicates alpha adrenergic vascular stimulation in the mechanism of these early hemodynamic changes of endotoxemia. Furthermore, the pattern of systemic arterial pressure in the animals pretreated with phenoxybenzamine was significantly different from that of the control group; the abrupt decline in arterial pressure did not

occur in the group which received phenoxybenzamine, although there was a progressive profound fall in the pressures over 30 minutes. This is consistent with the explanation offered by the Minnesota group[28] that splanchnic vascular pooling is responsible for the acute development of hypotension during endotoxin shock in dogs. In addition, the reports that phentolamine induces a similar response to endotoxin in the circulation of the stomach[17] and kidney[21] during endotoxemia support our findings.

Systemic arterial hypotension after injection of endotoxin was nearly abolished in a majority of dogs pretreated with the beta adrenergic receptor inhibitors, Nethalide and DCI, despite the occurrence of typical splanchnic vascular changes. This suggests a possible role for beta receptor stimulation in endotoxin shock, perhaps by endotoxin itself, pressor cathecholamines, or an unidentified depressor catecholamine. Another explanation which might be considered relates to the striking parallelism in the hemodynamic effects of epinephrine and endotoxin. Both compounds induce splanchnic pooling,[28-30] a diminished circulating blood volume,[23,31] splanchnic,[1,32] renal,[20,33] and cutaneous[14,32] vasoconstriction, and dilate the circulatory beds in the heart,[34,35] with an over-all reduction in total peripheral resistance.[36,37] Furthermore, plasma levels of epinephrine are increased early in endotoxin shock, with a less marked effect on the levels of norepinephrine.[8] The beta receptor antagonists enhance the pressor effects of epinephrine,[38] thereby lessening the difference between the vasoactive actions of epinephrine and norepinephrine. The over-all effect of Nethalide or DCI could, therefore, be an increase in total peripheral resistance in response to epinephrine, the major catecholamine released in the early phase of endotoxemia. This could explain the paradox of an amelioration of systemic arterial hypotension in the face of splanchnic vasoconstriction observed in the dogs to which Nethalide or DCI was administered.

Since the starting systemic arterial blood pressure in animals treated with phenoxybenzamine, Nethalide, DCI, antihistaminics, and Cyproheptadine was considerably lower than in control animals, a series of dogs whose arterial pressures were decreased by partial exsanguination was used as a control hypotensive group. In these animals the early response of systemic arterial pressure to endotoxin was diminished, and the depressor response to isoproterenol was also diminished. However, it is probable that partly exsanguinated dogs would exhibit a somewhat different initial response to the shocking effects of endotoxin, since plasma levels of cathecholamines would be elevated (as the extremely high splenic vascular resistance in the exsanguinated dogs indicates), and plasma levels of steroids would be increased just before the administration of endotoxin. Furthermore, the groups of dogs receiving antihistaminics, Nethalide, and DCI, with comparable initial blood pressures, exhibited different responses to endotoxin. At 10 minutes after endotoxin the declines in pressure were -21, -6, and -5 mm.Hg in these 3 groups, respectively. This suggests that the amelioration of endotoxin-induced hypotension observed in dogs treated with Nethalide, DCI, and phenoxybenzamine was due in large part to the pharmacologic agent used.

It is difficult to explain the diminished response of perfusion pressure to endotoxin in animals pretreated with Cyproheptadine and antihistamines, and the reduced portal venous changes in animals receiving Compound 48/80. Other substances which oppose the actions of serotonin and histamine have also been reported to change certain of the vascular responses to endotoxin.[39,40] Pretreatment of dogs with reserpine, Compound 48/80, atropine, morphine, and chloralose, and antihistaminic drugs did not diminish the hypotensive response to endotoxin. Although reserpine is a potent postganglionic adrenergic blocking agent[41] and depletes tissues of catecholamines and serotonin, studies in this laboratory indicate that measurable concentrations of catecholamines in the plasma are present in reserpine-treated dogs, and that endotoxin can induce elevations of the levels of epinephrine in the plasma of these animals. In addition, the increase in renal vascular resistance induced by endotoxin is not blocked by reserpine.[21] Compound

48/80 is a potent histamine liberator; however, in studies in progress, no difference has been observed in the concentrations in plasma of catecholamines, histamine, or serotonin between control animals and dogs pretreated with Compound 48/80. Furthermore, the animals treated with Compound 48/80 exhibit changes in these plasma neurohumoral agents in response to endotoxin which do not differ from those of control dogs. It appears that reserpine and Compound 48/80 are not totally effective agents in depleting body stores of neurohumoral substances. The specificity of atropine for the parasympathetic system, and the essential lack of difference between atropine-treated animals and dogs anesthetized with either pentobarbital or morphine and chloralose implies a minor role for cholinergic vascular receptors in the early phase of endotoxin shock.

There are two possible explanations for the apparent discrepancy between our findings and the report by Tsagaris and associates[40] in which they showed that antihistaminics effected some amelioration of the hypotension induced by lethal doses of *Escherichia coli* endotoxin. First, it may be that the endotoxin of *Escherichia coli* induces hypotension through activation of neurohumoral systems different from those stimulated by *Shigella flexneri* endotoxin. Second, the explanation could relate to the amount of endotoxin injected. According to Spink (personal communication), plasma concentrations of histamine are markedly elevated within 1 minute after the injection of amounts of endotoxin in excess of the lethal dose. We have not found increases in plasma histamine values after sublethal amounts of *Escherichia coli* endotoxin (unpublished observations). It is possible that a histamine component is superimposed on the basic effects of endotoxin when massive doses are injected, and under these conditions antihistaminics might exert partial attenuation of the hemodynamic effects of endotoxin.

Although the foregoing discussion has underscored the participation of catecholamines in endotoxin shock, the complexity of the pathologic state implies the interaction of many neurohumoral systems.

Summary

The effect of pharmacologic antagonists on vascular pressures in the perfused splenic artery, a branch of the portal vein, and in a systemic artery were monitored for 30 minutes after the injection of an LD_{50} of *Shigella flexneri* endotoxin. In control animals the injection of endotoxin was followed by a rapid increase in splenic arterial pressure, portal venous pressure, and vascular resistance across the spleen. Systemic arterial pressure exhibited an abrupt decline after the injection of endotoxin. Pretreatment of dogs with the alpha vascular receptor antagonist, phenoxybenzamine, prevented the increase in portal venous pressure and markedly attenuated the rise in splenic perfusion pressure. Pretreatment of dogs with either of two beta vascular receptor antagonists, Nethalide or DCI, ameliorated the fall in systemic arterial pressure without diminishing the splanchnic vascular responses to endotoxin. The serotonin antagonist, Cyproheptadine, diminished the increase in splenic perfusion pressure after endotoxin. Atropine, reserpine, Compound 48/80, or antihistaminics did not diminish the hypotensive response to endotoxin, although the increase in portal venous pressure was less marked in dogs to which Compound 48/80 had been administered, and perfusion pressure did not increase as markedly in animals pretreated with antihistaminics.

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